PATENT 674523-2012



It is respectfully requested that the application be amended, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as follows:

### **IN THE DRAWINGS:**

Please amend the drawings, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents as follows:

Please amend figures 1, 2, 4, 5, 6, 7, 8, 20, 21 and 26 as shown on the accompanying redlined copies.

# **IN THE SPECIFICATION:**

Please amend the drawings, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents as follows:

Please accept the enclosed substitute specification in place of the specification originally filed.

Page 65, line 1, please rewrite the paragraph thereat as follows:

Cassette 1- Translation initiation signal and signal peptide

In order to achieve correct translation initiation and secretion from mammalian cells, the following sequence is used (SEQ ID NO 16):

aagettCCACCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGT GTCCACTCC (SEQ ID NO: 38)

This contains a convenient *Hind*III restriction site for cloning into expression vectors (lower case), the consensus translation initiation signal for mammalian cells (ANNATGPu) and the coding sequence for a signal peptide sequence from an immunoglobulin gene.

Page 70, line 6, please rewrite the paragraph thereat as follows:

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The flexible linker, used to join the extracellular domain of B7.1 and the ScFv, was constructed by annealing two homologous oligonucleotides with engineered 5' Sma I and 3' Spe I sites - using oligonucleotides

Upper (SEQ ID NO: 6)

5' GGG GGT GGT GGC GGT GGT GGC GGC AGT GGC GGC GGC AG 3'

N.E.

and lower (SEQ ID NO: 16)

5' CTA GTT CCG CCG CCG CCA CTG CCG CCA CCA CCG CTC CCA CCA CCC CC 3'

The linker is cloned into pBluescript (Stratagene) via Sma I and Spe I to produce pLINK. The signal peptide (sp) and extracellular domain of murine B7.1 were amplified by PCR from pLK444-mB7.1 (supplied by R. Germain NIH, USA) via primers that introduce 5' EcoRI and 3' Sma I sites - primers forward

Page 70, line 18, please rewrite the paragraph thereat as follows:

The linker is cloned into pBluescript (Stratagene) via Sma I and Spe I to produce pLINK. The signal peptide (sp) and extracellular domain of murine B7.1 were amplified by PCR from pLK444-mB7.1 (supplied by R. Germain NIH, USA) via primers that introduce 5' EcoRI and 3' Sma I sites - primers forward (SEQ ID NO: 17)

5' C TCG AAT TCC ACC ATG GCT TGC AAT TGT CAG TTG ATG C 3'

reverse (SEQ ID NO: 18)

5' CTC CCC GGG CTT GCT ATC AGG AGG GTC TTC 3'

The B7.1 PCR product was cloned into pLINK via Eco RI and Sma I to form pBS/B7Link.

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The V<sub>H</sub> and V<sub>L</sub> of the 5T4 specific ScFv was amplified via primers -

forward primer (SEQ ID NO: 19)

5' CTC ACT AGT GAG GTC CAG CTT CAG CAG TC 3'

reverse primer (SEQ ID NO: 20)

5' CTC GCG GCC GCT TAC CGT TTG ATT TCC AGC TTG GTG CCT CCA CC 3'

that introduce 5' Spe I and 3' Not I sites from pHEN1-5T4 ScFv. PBS/B7Link was digested with Spe I and Not I and ligated with the ScFv to form OBM 233 consisting of the sequence shown as SEQ ID No. 11: B7 Link ScFv sequence (Figure 5).

Page 73, line 5, please rewrite the paragraph thereat as follows:

The sequence encoding a translation initiation sequence and the human immunoglobulin kappa light chain signal peptide is synthesized as two complementary single stranded oligonucleotides which when annealed also contain an internal *Xho I* site at the 5' end and in addition leave a *Xba I* compatible 5' overhang and a *Pst I* compatible 3' overhang

ctagactcgagCCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC GAG GTC CAG ctgca (SEQ ID NO: 21)

and

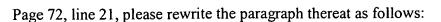
g CTG GAC CTC GGA GTG GAC ACC TGT AGC TGT TGC TAC CAA GAA GAG GAT GAT ACA GCT CCA TCC CAT GGTGGctcgagt (SEQ ID NO: 22)

This is then cloned into pBluescript II (Stratagene) restricted with Xba I and Pst I to create pBSII/Leader.

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The 5T4 ScFv is amplified by PCR from pHEN1 using oligonucleotides which incorporate a *Pst I* site at the 5' end of the product and a *Hind III* at the 3' end

GTC CAG CTG CAG CAG TCT GG (SEQ ID NO: 23)

and

CG TTT GAT TTC AAG CTT GGT GC (SEQ ID NO: 24)

This is then restricted with those enzymes and inserted into pBSII/Leader restricted with the same enzymes, creating pBSII/Leader/ScFv.

Page 74, line 1, please rewrite the paragraph thereat as follows:

The HIgG 1 constant region is amplified by PCR from the cloned gene using oligonucleotides which incorporate a *Hind III* site at the 5' end and a *Xho I* site at the 3' end

gege AAG CTT gaa atc aaa egg GCC TCC ACC AAG GGC CCA (SEQ ID NO: 25)

and

gege etegag TCA TTT ACC CGG AGA CAG GG (SEQ ID NO: 26)

This is then restricted with those enzymes and inserted into pBSII/Leader/ScFv restricted with the same enzymes, creating pBSII/Leader/ScFv/HG1. The sequence for this construct is shown in the Figure 4 (SEQ ID No 10).

Page 74, line 29, please rewrite the paragraph thereat as follows:

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This fusion construct is made by amplifying the human IgE1 constant heavy region by PCR cDNA derived from human B-cells RNA by RT and subsequently using oligonucleotides which incorporate a *Hind III* site at the 5' end and a *Xho I* site at the 3' end

gcgc AAG CTT gaa atc aaa cgg GCC TCC ACA CAG AGC CCA (SEQ ID NO: 27)

and

gcgc ctcgag TCA TTT ACC GGG ATT TAC AGA (SEQ ID NO: 28)

This is then restricted with those enzymes and inserted into pBSII/Leader/ScFv restricted with the same enzymes, creating pBSII/Leader/ScFv/HE1.

Page 76, line 1, please rewrite the paragraph thereat as follows:

Using cDNA derived by RT of RNA isolated from a cell line such the 293 human kidney line (ATCC: CRL1573), the DNA is amplified by PCR using oligonucleotides containing a *Spe I* restriction enzyme site at the N-terminus and a stop codon and a *Not I* site at the C-terminus

GG ACT AGT AAT AGT GAC TCT GAA TGT CCC (SEQ ID NO: 29)

And

ATT AGC GGC CGC TTA GCG CAG TTC CCA CCA CTT C (SEQ ID NO: 30)

The resulting product is digested with those enzymes and ligated to pBS/B7 Link which has been restricted with the same enzymes creating pBS/B7 Link EGF. The B7 Link EGF cassette is then excised with *Eco RI* and *Not I* and inserted into a derivative of pHIT111 (Soneoka *et al*, 1995, Nucl Acid Res 23; 628) which no longer carries the *LacZ* gene.

Page 82, line 31, please rewrite the paragraph thereat as follows:

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For B7-5T4 scFv the primers are as follows:-

Primer 1. B7-Sbf

ATCGCCTGCAGGCCACCATGGCTTGCAATTGTCAG (SEQ ID NO: 31)

Sbf I site = underlined

Kozak sequence = bold and italics with the ATG start codon underlined.

# Primer 2. 5T4sc-RI

GCGCGAATTCTTACCGTTTGATTTCCAGCTTGGT (SEQ ID NO: 32)

Eco RI site = underlined

TAA stop codon = bold and italics

The resultant product is then cloned into pONY 8.1 SM to produce the fusion protein construct shown in Figure 19a.

#### For L-5T4 scFv the primers are as follows:-

## Primer 1. L-Sbf

ATCGCCTGCAGGCCACCATGGGATGGAGCTGTAT (SEQ ID NO: 33)

Sbf I site = underlined

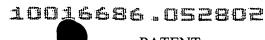
Kozak sequence = bold and italics, with the ATG start codon underlined.

#### Primer 2. 5T4sc-RI

GCGCGAATTCTTACCGTTTGATTTCCAGCTTGGT (SEQ ID NO: 34)

Eco RI site = underlined

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TAA stop codon = bold and italics

The resultant product is then cloned into pONY 8.1 SM to produce the construct shown in Figure 19b.

Please replace the previously filed sequence listing with the enclosed papers titled -- Sequence Listing.--

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